REMARKS

Status of the Claims

Claims 1, 2, 3, and 5 are pending in the present application. Claim 4 was previously canceled. Claim 1 is allowed. Claims 2 is amended substantially as suggested by the Examiner in the April 13, 2010 interview. Claim 3 is redrafted in independent form and is amended for clarity. Support for amended claim 3 is found in pending claim 2. No new matter is added by way of this amendment. Reconsideration is respectfully requested.

Statement of the substance of the interview

Applicants and Applicants' representative thank the Examiner for initiating an interview on April 13, 2010, for the purpose of expediting prosecution, although no agreement was reached. Applicants submit that the substance of the interview is substantially as described in the interview summary, which was issued on May 3, 2010.

Claim Objections

Claim 3

Claim 3 is objected to because of the recitation "[a] threonine-producing *Corynebacterium* strain prepared by the method as set forth in claim 2", *see* Office Action, page 2. The Examiner objects to this recitation because claim 2 describes "a method for increasing the yield of threonine produced by a theronine-producing *Corynebacterium* strain" and not a method for preparing a threonine-producing *Corynebacterium* strain.

In view of the amendment to claim 3, Applicants believe the objection is overcome and respectfully request withdrawal.

Claim 5

The Examiner also objects to claim 5, *see* Office Action, page 3. According to the Examiner, claim 5 is in improper form for failing to limit the subject matter of claim 2. The Examiner states that a threonine-producing *Corynebacterium* strain having an endogenous threonine importer gene comprising a continuous DNA sequence from the 1,772 base to the

3,025 base among DNA sequences with the SEQ ID NO: 1 *must* be a *Corynebacterium glutamicum*, *emphasis added*.

Applicants submit that claim 2 is not limited to naturally occurring *Corynebacterium*, but may also encompass recombinant species that endogenously express the threonine importer gene from nucleotides 1,772 to 3,025 of SEQ ID NO: 1. Accordingly, species other than *Corynebacterium glutamicum* may comprise nucleotide bases 1,772 to 3,025. Therefore, claim 5 properly limits claim 2. In view of the foregoing, withdrawal of the rejection is respectfully requested.

Issues Under 35 U.S.C. § 112, Second Paragraph

Claim 2 is rejected as allegedly unclear, *see* Office Action, pages 3-4. Claim 2 is amended substantially according to the Examiner's suggestion in the interview of April 13, 2010. Accordingly, Applicants believe the rejection is overcome and respectfully request withdrawal.

Issues Under 35 U.S.C. § 103

Claims 2, 3, and 5 are rejected under 35 U.S.C. § 103(a) as allegedly obvious over Palmieri *et al.*, *Arch. Microbiol.*, 1996, 165:48-54, ("Palmieri"), in view of U.S. Publication No. 2002/0197605 to Nakagawa *et al.*, ("Nakagawa"), U.S. Patent No. 6,696,561 to Pompejus *et al.*, ("Pompejus"), and Simic *et al.*, *Journal of Bacteriology*, 2001, 183:5317-5324, ("Simic"), *see* Office Action, pages 5-8.

Basis for the Rejection

The Examiner alleges that Palmieri describes a threonine uptake carrier that transports threonine in symport with sodium ions. According to the Examiner, Palmieri further teaches that excess serine can inhibit threonine uptake. The Examiner admits that Palmieri does not describe inhibiting threonine uptake by inactivating the endogenous threonine importer gene comprising a continuous DNA sequence from base 1772 to base 2025 of SEQ ID NO:1 in a threonine-producing *Corynebacterium*. Nevertheless, the Examiner believes that Nakagawa, Pompejus, and Simic remedy this deficiency.

According to the Examiner, Nakagawa and Pompejus disclose isolated sequences encoding for a proton/glutamate symporter, which are similar to those described in the instant claims. The Examiner believes that an ordinary artisan at the time of the invention would have inhibited the threonine uptake carrier described in Palmieri by inactivating a coding region of the proton/sodium-glutamate symport protein gene, as described by Nakagawa and Pompejus, by constructing insertion and/or deletion mutants as described in Simic. According to the Examiner, an ordinary artisan would have been motivated to modify Palmieri's method of inhibiting the threonine uptake carrier because an ordinary artisan would have recognized that the proton/sodium-glutamate gene that is described in Nakagawa and Pompejus is likely to be involved in theonine uptake. Accordingly, by inhibiting the sequences described in Nakagawa and Pompejus by using insertion and/or deletion mutants, an ordinary artisan would have inactivated the sequences described in the present claims.

The Examiner has failed to establish a prima facie case of obviousness

Applicants submit that the Examiner has failed to establish a *prima facie* case of obviousness. The Federal Circuit held in *In re Kubin* 561 F.3d 1351 (Fed. Cir. 2009) that it is impermissible to reject claims based upon an "obvious to try" rationale where what was "obvious to try" was to explore a new technology or a general approach that seemed to be a promising field of experimentation, but where the prior art gave only general guidance as to how to achieve the claimed invention.

Applicants submit that the cited references only give general guidance, if any guidance, regarding how to achieve the claimed invention. Palmieri and Simic indirectly demonstrate the presence of a threonine importer gene in a *Corynebacterium* strain. Nevertheless, neither of these references teaches the specific threonine importer gene sequences described in the instant claims. Palmieri further teaches that inhibiting threonine influx with excess serine increases the rate of threonine production, *see* Fig. 5 of Palmieri. However, this teaching merely shows that threonine and serine uptake is achieved *via* the same uptake system. Accordingly, these references do not suggest knocking out the specific gene sequences described in the present claims to enhance threonine production.

Nakagawa and Pompejus describe nucleic acid sequences that assertedly encode a membrane transport protein from a *Corynebacterium* strain. However, the membrane transport

capacity of the encoded protein is merely assumed by Nakagawa and/or Pompejus and is not substantiated by actual experimentation. Further, an ordinary artisan could not have been reasonably certain from Pompejus that SEQ ID NO: 543, which assertedly encodes a proton/sodium-glutamate symport protein, is a threonine importer gene because this function was also not established by actual experimentation. In addition, the proton/sodium-glutamate gene sequences described in the cited reference are not identical to the DNA sequence described in the instant claims.

The Examiner states that Palmieri teaches that threonine is co-transported with Na+ and that this disclosure would have prompted an ordinary artisan to inactivate the sequences in Palmieri's *Corynebacterium* strain that correspond to the proton/sodium-glutamate gene sequences described in Nakagawa or Pompejus since an ordinary artisan would have believed that the proton/sodium gene sequences were involved in threonine uptake. At best, however, Palmieri teaches that a threonine uptake system may be involved in threonine production, *i.e.*, Palmieri describes a promising field of experimentation. Palmieri does not particularly suggest that the proton/sodium gene sequences described in Nakagawa or Pompejus are involved in the threonine uptake system.

Furthermore, the instant invention may not have been achieved even if Palmieri were modified in the manner described by the Examiner. An ordinary artisan would have had to obtain numerous mutants to find one with the inactivated sequences described in the present claims.

In view of the foregoing, the cited references provide only general guidance regarding the presence of a threonine importer gene, and do not provide the more specific guidance that is needed to establish a *prima facie* case of obviousness. Accordingly, the Examiners rationale for suggesting that an ordinary artisan would have been motivated to inactivate the sequence described in the instant claims to increase threonine production in *Corynebacterium* is based upon little more than an improper "obvious to try" rationale.

Advantages of the claimed invention

Applicants further submit that the claimed invention results in advantages in comparison to prior art methods for increasing threonine production. Applicants initially note that the instant inventors identified the threonine importer gene by actual experimentation. Accordingly, it was not until Applicants' invention that the specific sequence responsible for threonine importation in *Corynebacterium* was actually known. Moreover, Applicants discovered that when the L-threonine importer gene of a *Corynebacterium* strain is inactivated, the yield of L-threonine increases since threonine import into a cell is essentially blocked.

Moreover, the present invention achieves a substantial increase in threonine production in a cost effective manner. For instance, Example 5 of the originally filed application demonstrates that a parent strain accumulated 7.3 g/l of threonine in the culture medium while a thrI defective strain accumulated 8.1 g/l of threonine, which is an increase of approximately 10%. In contrast to Applicants' method of achieving increased threonine production, Palmieri's method for producing threonine in excess is costly and uneconomical.

In addition, Applicants submit herewith Exhibit 1, which also demonstrates a substantial increase in threonine production according to the claimed invention. Exhibit 1 provides experimental data, which demonstrate that after depletion of glucose, the concentration of threonine in the culture of a parent strain, *C. glutamicum* CJT-11, is reduced. This reduction is due to importation of threonine from the culture into the cell, which is degraded. In contrast, the concentration of threonine released from *C. glutamicum* CJT-11, which is a threonine importer gene (thrY) defective strain, according to the claimed invention, is increased since threonine is blocked from importation into the cell.

In view of the foregoing, the claims are also not rendered obvious by the cited references due to the above-described superior advantages of the claimed invention. Accordingly, withdrawal of the rejection is respectfully requested.

CONCLUSION

In view of the above amendment and remarks, Applicants believe the pending application is in condition for allowance.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Linda T. Parker, Ph.D., Registration No. 46,046 at the telephone number of the undersigned below to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Director is hereby authorized in this, concurrent, and future replies to charge any fees required during the pendency of the above-identified application or credit any overpayment to Deposit Account No. 02-2448.

Dated: AUG 0 3 2010

Respectfully submitted,

By _____

Craig A. McRobbie
Registration No.: 42874
BIRCH, STEWART, KOLASCH & BIRCH, LLP
8110 Gatehouse Road, Suite 100 East
P.O. Box 747
Falls Church, VA 22040-0747
703-205-8000

Attachments

EXHIBIT 1

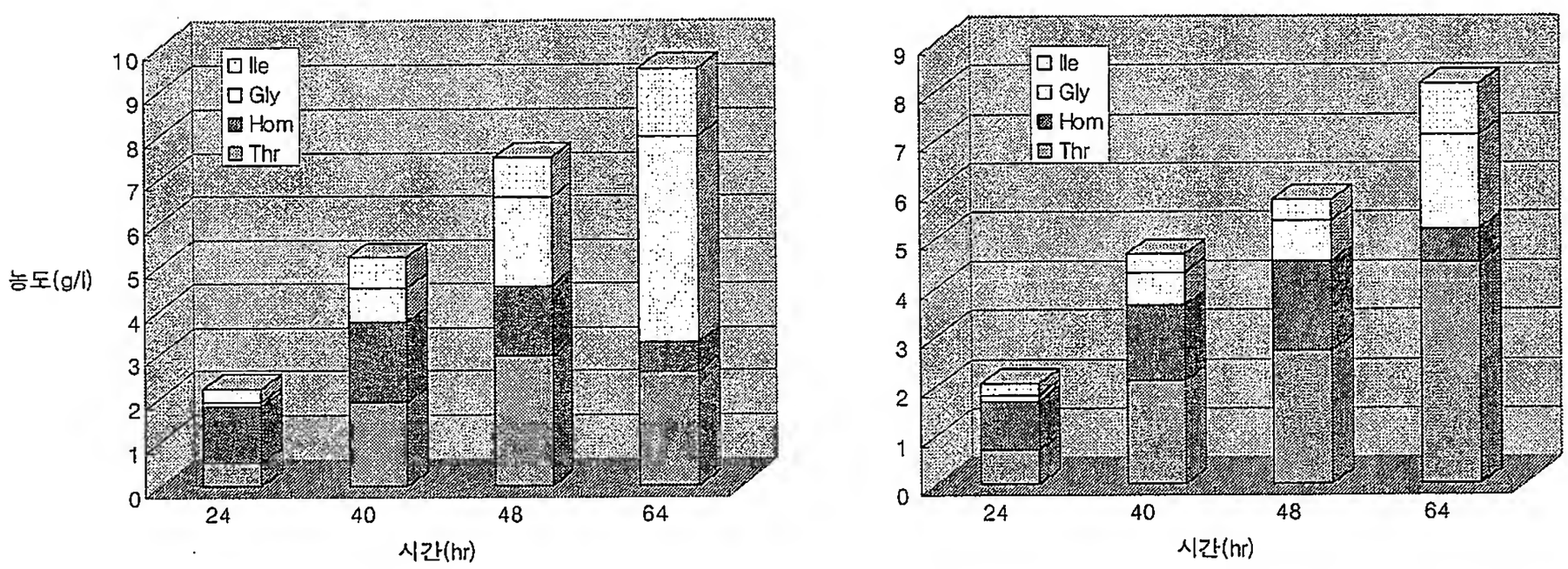
A NOVEL L-THREONINE IMPORTER FROM CORYNEBACTERIUM AND A PREPARATION METHOD OF A STRAIN PRODUCING L-THREONINE

Purpose of the experiment

Purpose is the increase of the accumulated concentration of the produced/released threonine in culture media by blocking threonine import into cell.

Summary of the experiment

- In order to produce threonine importer gene defective strain, named *C. glutamicum* CJT-11, *thrY* (NCgl2924) gene(threonine importer gene) of recombinant *C. glutamicum* CJT-10 which designed for producing threonine is defective(inactivate).
- For incubating parent strain(*C. glutamicum* CJT-10) and *thrY* defective strain(*C. glutamicum* CJT-11), the level of threonine, homoserine, glycine, isoleucine is estimated.



[Figure] Accumulated concentration of amino acids in culture media (X-axis: concentration(g/l), Y-axis: time(hr))

Left – amino acid profile of the broth of *C. glutamicum* CJT-10

Right - amino acid profile of the broth of *C. glutamicum* CJT-11

Result

- As shown in figure, in case of the parent strain (*C. glutamicum* CJT-10), the accumulated concentrations of threonine and homoserine are increased before 48hr, and then the accumulated concentrations of threonine and homoserine are

reduced and the accumulated concentrations of isoleucine and glycine which are degradation product of threonine are increased after 48h.

- In case of *thrY* defective strain(*C. glutamicum* CJT-11), the accumulated concentration of threonine is continually increased .
- Finally, for 64 hr incubating duration, the yield of threonine of *C. glutamicum* CJT-11 is increased about 73% compared with that of *C. glutamicum* CJT-10 (2.6 -> 4.5 g/l).

Discussion

Threonine import into the cell can be controlled(blocked) by inactivating threonine importer gene of *Corynebacterium glutamicum.*